23 NOV. 1928

CONSIDERATIONS ON THE FUNGI FOUND IN BLASTOMYCOSIS

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The term "blastomycosis" covers all diseases due to "yeast-like" fungi, but in practice in this country it is generally restricted by most dermatologists to indicate a clinical entity or group of closely allied entities characterized by the presence of granulomatous, papillomatous or framboesiform lesions in which certain yeast-like fungi are found. These fungi have been classified in various manners. Some authorities maintain that there is only one fungus causing blastomycosis; other observers are of the opinion that there are two species: *immitis* and *dermatitidis* (Gilchristi). These two species have been placed in the same genus or in different genera. Several authorities place *immitis* in the genus *Coccidioides,* a genus belonging to the Ascomycetes, and *dermatitidis* in the genus *Blastomyces* or in the genus *Cryptococcus,* or in the genus *Oidium* belonging to the Fungi Imperfecti.

My researches during the last few years have led me to believe that there are a

[Reprinted from *American Medicine,* New Series, Vol. XXIII, No. 5, pp. 289-295, May, 1928.]

plurality of species of the fungi causing blastomycosis. By using special media (mannitol and lactose agar) three types can be separated fairly easily: *immitis, dermatitidis, tulanensis.* These fungi according to my recent observations seem to belong all to the Fungi Imperfecti and to the same genus. As, unfortunately for botanical reasons, the genus *Blastomyces* cannot stand, I have suggested the creation of a new genus: *Blastomycoides.*

Genus Blastomycoides, Castellani, 1926

Synonyms.—Blastomyces, Oidium pro parte, Cryptococcus pro parte, Coccidioides pro parte, Zymonema pro parte.

Remarks.—As for botanical reasons the generic term *Blastomyces* cannot stand, I suggested recently the creation of a new genus, *Blastomycoides,* which should be placed in the Family Oosporaceæ rather than in the Family Cryptococcaceæ.

Definition.—The genus *Blastomycoides* may be defined as follows: "Oosporaceæ, with abundant mycelium and aerial hyphæ when grown on glucose agar; abundant mycelium with, at times, lateral conidia when grown in glucose broth hanging drops; no gas fermentation of any sugar. In the lesions, only roundish or oval large cells are seen, at times budding, with well-defined double contour and well-marked

granulations in the plasma present, without any mycelium." This genus in all probability contains a number of species, but for the time being the following may be differentiated: *Blastomycoides immitis, Blastomycoides dermatitidis, Blastomycoides tulanensis.*[1] For the practical differentiation of these three types I have found useful to grow the fungi on mannitol agar (1 per cent.) and on lactose agar (1 per cent.), besides other media. The agar is made up with peptone water, not broth. Large tubes should be used, not the ordinary tubes used for bacterial cultures. The inoculated tubes are kept at 26° for two or three weeks and then examined. In a fairly warm climate like New Orleans keeping the tubes at the temperature of the room is satisfactory.

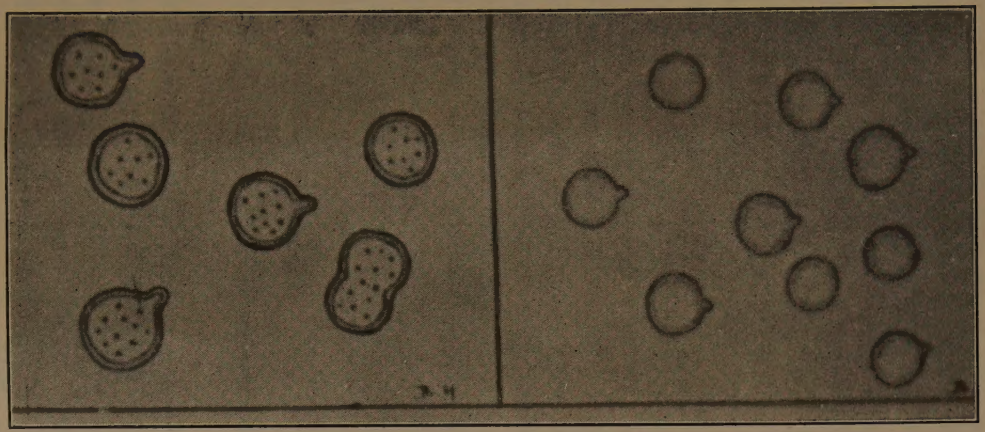
Blastomycoides immitis, Rixford and Gilchrist, 1897

Synonyms.—Coccidioides pyogenes, Rixford and Gilchrist, 1896; *Oidium coccidioides,* Ophüls, 1905; *Oidium protozoides,* Ophüls, 1905; *Posadasia esferiformis,* Canton, 1898; *Oidium immite,* Verdun, 1907; *Oidium pyogenes,* Verdun, 1906; *Mycoderma immitis,* Brumpt, 1925.

[1] A fourth type might perhaps be separated producing black colonies in glucose agar and practically every other medium: B. niger cast. I have only one strain of this type and the investigation of it has not yet been completed.

Remarks.—For this fungus the genus *Coccidioides* was created by Rixford and Gilchrist in 1897. The principal characteristic would be the presence of very large asci with numerous ascospores, the so-called "cysts" of some observers. The botanical investigation I have carried out on two strains of this fungus did not show the presence of true ascospores. The numerous minute bodies described as ascospores inside the large round cells are not, in my opinion, ascospores; they are protoplasmatic granules or spherules. My contention seems to be proved by the fact that when one of the large roundish cells produces a bud which later becomes a mycelial filament the same minute bodies are seen in the bud and in the mycelium so produced. I venture to state, therefore, that for the present at least this fungus should not be considered to belong to the Ascomycetes but to the Fungi Imperfecti and being very similar to *Blastomycoides dermatitidis* should be placed in the same genus, *viz.,* in the genus *Blastomycoides.*

Characters of the Fungus in the Lesions and in Cultures.—In the lesions large roundish or oval spore-like bodies are seen, 10 to 20 microns and even much larger. They have a well-defined double contour and numerous roundish bodies are seen



BLASTOMYCETOID TYPE. CRYPTOCOCCOID TYPE.

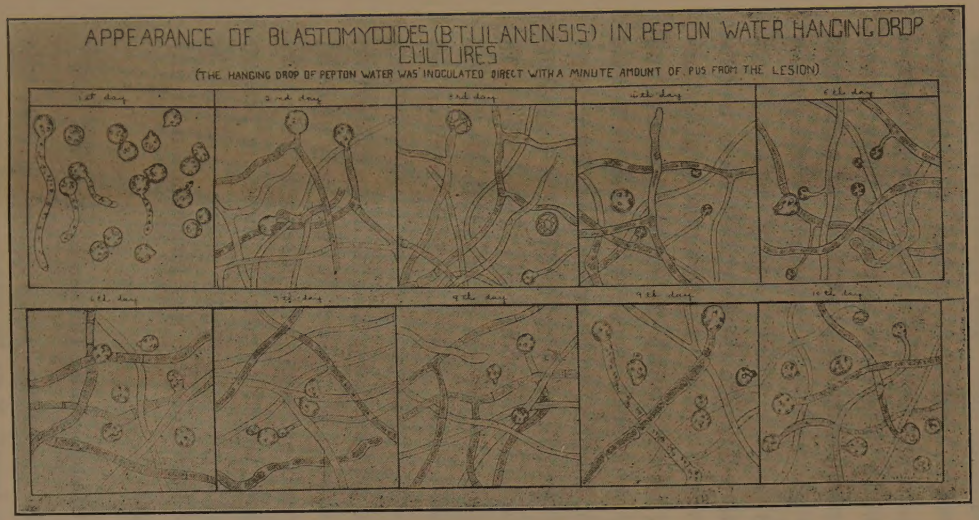
Blastomycetoid Type and Cryptococcoid Type of Yeast-Like Organisms.

in the protoplasm which I am inclined to consider to be protoplasmatic granules or spherules. On mannitol agar 1 per cent. after two weeks incubation at 26° but as a general rule also after the same period of incubation at 22° or at the room temperature the growth becomes of a black slate color and most of the medium takes the same color. On glycerol agar the growth is white-greyish with a fluffy surface with a somewhat woolly appearance. On lactose agar the growth is whitish or greyish, there is no evident darkening of the medium. On potato it is usually white and fluffy. On carrot it is also white and fluffy. In glucose broth the fungus growth is both at the surface and bottom. In hanging drop cultures a large amount of mycelial threads are seen, lateral conidia supported by sterigmata are often absent or present only in small numbers.

Blastomycoides Dermatitidis, Gilchrist and Stokes, 1898

Synonyms. — *Blastomyces dermatitidis,* Gilchrist and Stokes, 1898, *Oidium dermatitidis,* Ricketts; *Cryptococcus gilchristi,* Vuillemin; *Zymonema gilchristi,* de Beurmann and Gougerot; *Mycoderma gilchristi,* Jannin, 1913.

Characters of the Fungus in the Lesions and in Cultures.—In the lesions large


APPEARANCE OF BLASTOMYCOIDES (BTULANENSIS) IN PEPTON WATER HANGING DROP CULTURES
(THE HANGING DROP OF PEPTON WATER WAS INOCULATED DIRECT WITH A MINUTE AMOUNT OF PUS FROM THE LESION)
1st day
2nd day
3rd day
4th day
5th day
6th day
7th day
8th day
9th day
10th day

Appearance of Blastomycoides Tulanensis in hanging drops cultures of various age. The hanging drop (peptone water) was inoculated direct with a minute portion of pus.

roundish cells are found, 10 to 16 microns and much more, with a well-marked double contour and well-marked protoplasmatic granules. On glucose agar the growth is whitish or greyish and often fluffy; abundant mycelium is present. In mannitol agar (1 per cent.) the growth is white-greyish with often a peculiar woolly surface. After a time (two to three weeks) there usually develops a black pigmentation or a darkening of the growth and of the medium and the same phenomenon takes place in lactose agar cultures. In glycerine agar (1 per cent.) the growth is white, fluffy. On potato the growth is abundant, with white duvet. In glucose broth and other sugar broths the growth takes place all over the medium, including the surface portion. In glucose broth and peptone water hanging drops made from glucose agar cultures, at times only mycelial threads are seen, with absence or only very scanty lateral conidia; if these are present they seem to be mostly sessile. Gelatine is not liquified. No gas fermentation of any sugar.

Blastomycoides Tulanensis, Castellani, 1926

In the lesions large roundish or oval cells are found, 8 to 16 microns and even very much larger with well-marked double contour and some granules or spherules in the

protoplasma of fairly large size. On glucose agar the growth is white and somewhat fluffy, this being due to tufts of aerial hyphæ. In mannitol agar the growth is white and the medium does not become darkened. On lactose agar the growth is white; there is no darkening of the medium. In glycerol agar the growth is at times nodular with slight white duvet, at times fluffy white. In peptone water or glucose broth hanging drop cultures made from glucose agar cultures, round lateral conidia supported by well-developed sterigmata are usually present. In cultures on solid media, especially in the submerged portion of the growth, at times large round or oval cells with a well-marked double contour membrane and protoplasmatic granulations may be present very similar or identical to those found in the lesions.

Blastomycoides tulanensis on glucose agar and several other media often grows like a trichophyton with white duvet and at times radiating furrows.

In the following table are collected some differential cultural characters of the three types of *Blastomycoides*. On glucose agar the differences are slight or absent, but they are usually distinct on mannitol, lactose and glycerol. The tubes were kept at 26° C. for three weeks.

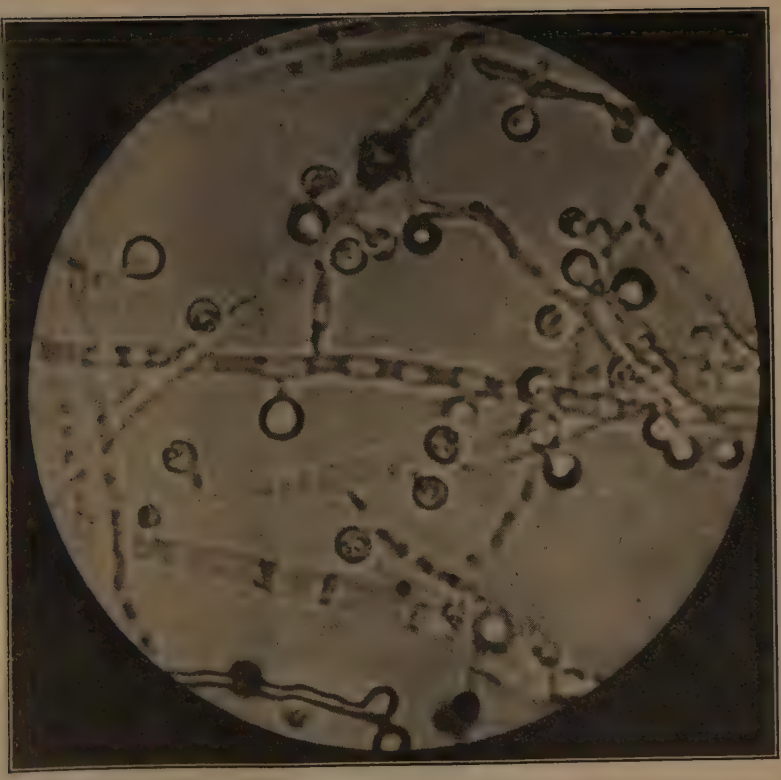
Blastomycoides Dermatitis (hanging drop culture four days old).

	Glucose agar	*Mannitol agar*	*Lactose agar*	*Glycerol agar*
B. immitis	Whitish-greyish or light fawn	Growth and medium slate black color	White or greyish	Greyish, woolly appearance
B. dermatitidis	Whitish or greyish, fluffy White, fluffy	Dark brown or black pigmentation of the growth and medium	Black pigmentation with darkening of medium	White, fluffy
B. tulanensis		Always white, no darkening of medium	White, medium not darkened	At times fluffy, at times nodular with short white duvet

Remarks on Yeast-like Fungi in General.—The Blastomycoides fungi belong to that large group of fungi which are generally known as "yeast-like fungi." The term "yeast-like fungi" or "budding fungi" is unscientific, but useful in practice. "Yeast-like fungi" are fungi which in the lesions appear as free oval or roundish cells, some of them budding, with usually no mycelium at all, exceptionally a few mycelial elements. In cultures there may be only budding cells, or budding cells and mycelium or practically mycelium only. Morphologically, in the lesions two principal types of yeast-like fungi may be distinguished: (a) the *blastomycetoid* (*blastomycetic*) type, (b) the *cryptococcoid* (*saccharomycetic*) type.

The characters of the blastomycetoid type are the following: Cells large, double contour very well marked, granules or spherules in the protoplasm very evident and of comparatively large dimensions. The characters of the cryptococcoid (or saccharomycetic) type, are the following: Cells generally smaller, double contour absent or much less marked, protoplasmatic granules not so evident and usually much finer.

These fungi belong to widely different botanical groups; when cultivated, some of



Blastomycoides Tulanensis (hanging drop culture four days old).

them show the same morphologic characters as in the lesions, *viz.*, oval or roundish budding cells, with no mycelium, but quite a number of them show presence of mycelium, and some, in fact, mycelium only.

Botanical Classification of Yeast-like Fungi (Budding Fungi).—The late Dr. Chalmers and myself have given a botanical classification of the organisms in the third edition of our "Manual of Tropical Medicine." Some of them are ascomycetes, others are fungi imperfecti; the principal families to which they belong are the following:

a. In cultures, budding cells, and in addition asci and ascospores present, no mycelium—Family Saccharomycetaceæ, Rees, 1870.

b. Same as a, but mycelium present—Family Endomycetaceæ, Rees.

c. In cultures, budding cells (blastospores) present, no asci and no mycelium—Family Cryptococcaceæ, Kützing, 1833.

d. Same as c, but mycelium present—Family Oosporaceæ, Saccardo.

Each family contains a number of genera, a brief description of which may be found in Castellani and Chalmers' "Manual of Tropical Medicine," and also in the Gehrmann Lectures, University of Illinois, 1926 (Fungi and Fungous Diseases, by Aldo Castellani).

Simple Classification of Yeast-like Fungi (Budding Fungi) for the Use of the Clinician and Clinical Pathologist.—An elaborate botanical classification is not necessary for the purposes of the clinician and clinical pathologist, and the simple scheme shown in the following two tables may be of practical use to them.

Key to the Principal Groups of Yeast-like Fungi (Budding Fungi)

"Yeast-like Fungi"

(Fungi which in the lesions appear as round or oval budding cells, no mycelium or only exceptionally a trace).

Morphological Characters in the Lesions

Blastomycoid appearance, viz., large cells with well defined double contour and well marked granulations, Blastomycoides (Blastomyces).

Cryptococcoid appearance, viz., cells smaller with double contour absent or faint and granulations very fine or absent.

Cultures

Mycelium

absent in all media

Ascospores

absent: Cryptococcus

present: Saccharomyces

present in certain media

Ascospores

absent: Monilia

present: Endomyces

Simple Classification of Yeast-like Fungi (Budding Fungi)

- LARGE ROUNDISH CELLS WITH WELL MARKED GRANULATIONS AND WELL DEFINED DOUBLE CONTOUR, BLASTOMYCOIDES

- SMALLER WITH GRANULATIONS ABSENT OR VERY FINE, DOUBLE CONTOUR ABSENT OR FAINT (SO CALLED CRYPTOCOCCOID APPEARANCE)

CULTURES

- MYCELIUM ABSENT
  - ASCOSPORES ABSENT. CRYPTOCOCCUS

  - ASCOSPORES PRESENT: SACCHAROMYCES

- MYCELIUM PRESENT IN CERTAIN MEDIA
  - ASCOSPORES ABSENT: MONILIA

  - ASCOSPORES PRESENT: ENDOMYCES

Conclusion

The fungi found in Blastomycosis, using the term Blastomycosis in the limited sense, as usually done in this country, do not belong to one species only; they belong to several species. By using mannitol and lactose agar three types can be differentiated fairly easily: *immitis* type, *dermatitidis* (or *Gilchristi*) type, *tulanensis* type. After two to three weeks at 26° the cultures of the *immitis* type are slate black on mannitol, greyish on lactose; the cultures of *dermatitidis* are black on mannitol and lactose; the cultures of *tulanensis* are white both on mannitol and on lactose.

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CARBOL-FUCHSIN PAINTS IN THE TREATMENT OF CERTAIN CASES OF EPIDERMOPHYTOSIS

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For some time I have carried out experiments on the use of fuchsin paints in the treatment of epidermophytosis. The paint I generally use has the following formula: Saturated alcoholic solution of basic fuchsin, 10 c. c.; 5 per cent. aqueous carbolic solution, 100 c. c. Filter and add boric acid, 1 gram. After two hours add 5 c. c. of acetone. Two hours later 10 grams of resorcinol are added. The paint should be kept in a dark-colored bottle with a glass stopper.

The basic ingredient of this paint is the ordinary carbol-fuchsin in common use in bacteriologic laboratories for staining T. B. As a matter of fact the simple carbol-fuchsin gives good results, but the addition of boric acid and the acetone seems to increase its action and to make it more penetrating. The addition of resorcinol about 10 per cent. is useful in many cases.

[Reprinted from *American Medicine*, New Series, Vol. XXIII, No. 5, pp. 351-352, May, 1928.]

The paint is applied to the affected parts with a small wad of cotton wool or a small sponge daily, in some cases several times a day, or every other day or twice a week. If eczematoid symptoms are present and are very acute the paint may be diluted with the same amount of water, but usually it may be safely applied undiluted provided the eczematoid patches are of true epidermophytic origin.

Types of Epidermophytosis Treated.—I have used the paint principally in the following conditions:

1. *Chronic cases of epidermophytosis interdigitalis pedum.*—The result is generally satisfactory, especially cases with secondary pyogenic infection. At first when the associated pyogenic infection was very heavy and there was present diffuse redness with pustules and abrasions I used to advise the application of a saturated boric lotion (2 per cent.) or a boric-resorcin lotion, such as boric acid gr. xxx, resorcin gr. xxx, aquæ ad ℥ iv, for two or three days before starting the fuchsin paint. Recently, however, in several cases I have started the fuchsin treatment immediately with excellent results.

2. *Cases of pruritus ani and pruritus vulvæ of mycotic origin.*—As I have stated in other publications a fairly large number of cases of pruritus ani and pruritus vulvæ

are of mycotic origin; very often we are dealing with a latent epidermophytosis with superadded saccharomycetic, cryptococcus, monilial, and bacterial infections. In a number of these cases the paint acts well.

Illustrative Case.—Mrs. B. came to consult me in London last summer, complaining of unbearable vaginal and anal pruritus. On examination an eczematoid condition of the labia majora and of the perianal region was present; the patient complained of pruritus also in the labia minora and in the clitoris. The paint was applied freely not only to the external surface of the labia majora but also to the internal surface, and also to the labia minora and clitoris, at first diluted with equal part of water, then neat as it caused no smarting. The application was done at first every other day, then twice a week, then once a week. Within three weeks the condition was completely cured.

From this case, before starting the treatment, an epidermophyton (*E. rubrum*), was grown and in addition a monilia and various bacteria, also a most peculiar organism which I was uncertain at first how to classify, as certain forms of it resembled a cryptococcus, others a thick bacillus. I have described it elsewhere under the name *Bacillus multiformis.* (Proceedings of the Soc. for Exper. Biol. and Med., 1928.)

Cases of Epidermophytosis Inguinalis and Corporis Not Answering Satisfactorily to the Usual Routine Method of Treatment by Antimycotic Salves.—While most cases of tinea cruris answer well to the usual

routine treatment by salicylic-benzoic acid ointment (Whitfield's ointment) consisting of salicylic acid gr. xxx (℥i), benzoic acid gr. xxx (℥i), and vaseline 1 ounce, or the salicylic-sulphur ointment of the writer—ac. salicyl. gr. xxx (℥i), sulphur. praec. gr. xxx, vaseline ad ℥i—in a certain percentage of cases the result is unsatisfactory; in these cases I have found the paint useful.

Cases of Epidermophytosis Simulating Moist Eczema.—In these cases the paint seems to be very successful.

Post-epidermophytosis Pruritus.—In previous publications I have called attention to the occurrence of post-epidermophytosis pruritus, *viz.*, cases in which after the usual routine treatment all the objective symptoms of epidermophytosis disappear, but the pruritus remains for weeks and months. The pruritus may be of different origin: a, Altho the clinical signs have disappeared the mycotic infection is still present; b, overtreatment an mild hypersensitivity to the various antimycotic ointments; c, nervous origin; in some highly strung individuals the pruritus remains long after the infection has disappeared.

In the first group of cases the use of the carbol-fuchsin paint will be found useful.

Conclusion.—Some time ago I introduced the use of various fuchsin paints in the

treatment of epidermophytosis. The paint which apparently answers best is the following: Saturated alcoholic solution of basic-fuchsin, 10 c. c.; 5 per cent. aqueous carbolic acid solution, 100 c. c. Filter and add boric acid 1 gram; after two hours add 5 c. c. of acetone; and two hours later 10 grams of resorcinol may be added.

This paint with or without resorcinol, but apparently better with resorcinol, I find to be fairly successful in certain cases of epidermophytosis in which the routine treatment by the usual antimycotic ointments may, at times, give disappointing results, especially cases of epidermophytic pruritus ani, epidermophytic pruritus interdigitalis and in cases of epidermophytosis simulating moist eczema and also in cases of epidermophytosis complicated with pyogenic infections.

A disadvantage of using the paint is the deep red coloring of the skin, but patients who have had epidermophytosis for years and have tried all kinds of treatment do not object to it. Up until now cases of hypersensitiveness to the paint have not been found, but there is of course no doubt that if this method should become generalized such hypersensitive individuals will be found and it will therefore be advisable in cases of generalized epidermophytosis to use the paint at first only on a small patch.